FIGURES

Figure 1 - Structure of Ala-hGH and hGH

CORRECT HUMAN GROWTH HORMONE N-TERMINAL SEQUENCE CAN BE ACHIEVED BY THE *IN-VITRO* ENZYMATIC CLEAVAGE OF ALA-HUMAN GROWTH HORMONE

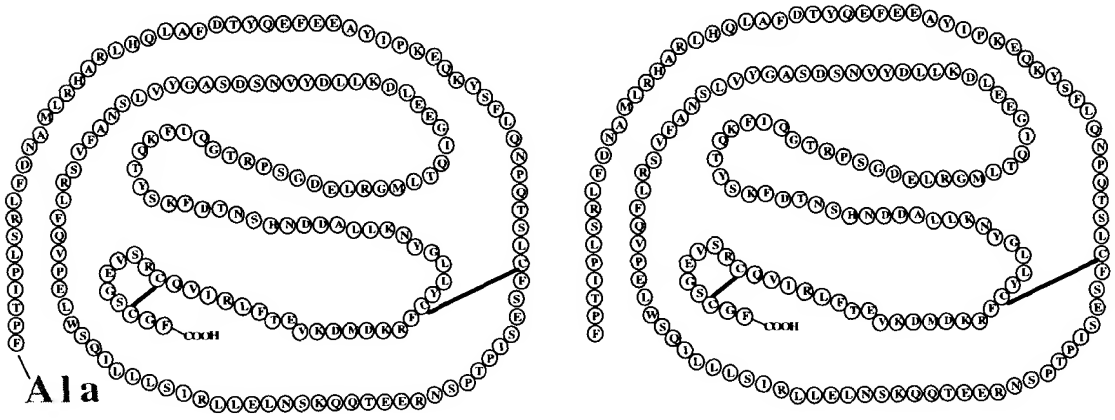


Figure 2 - Process for Production of hGH from Ala-hGH

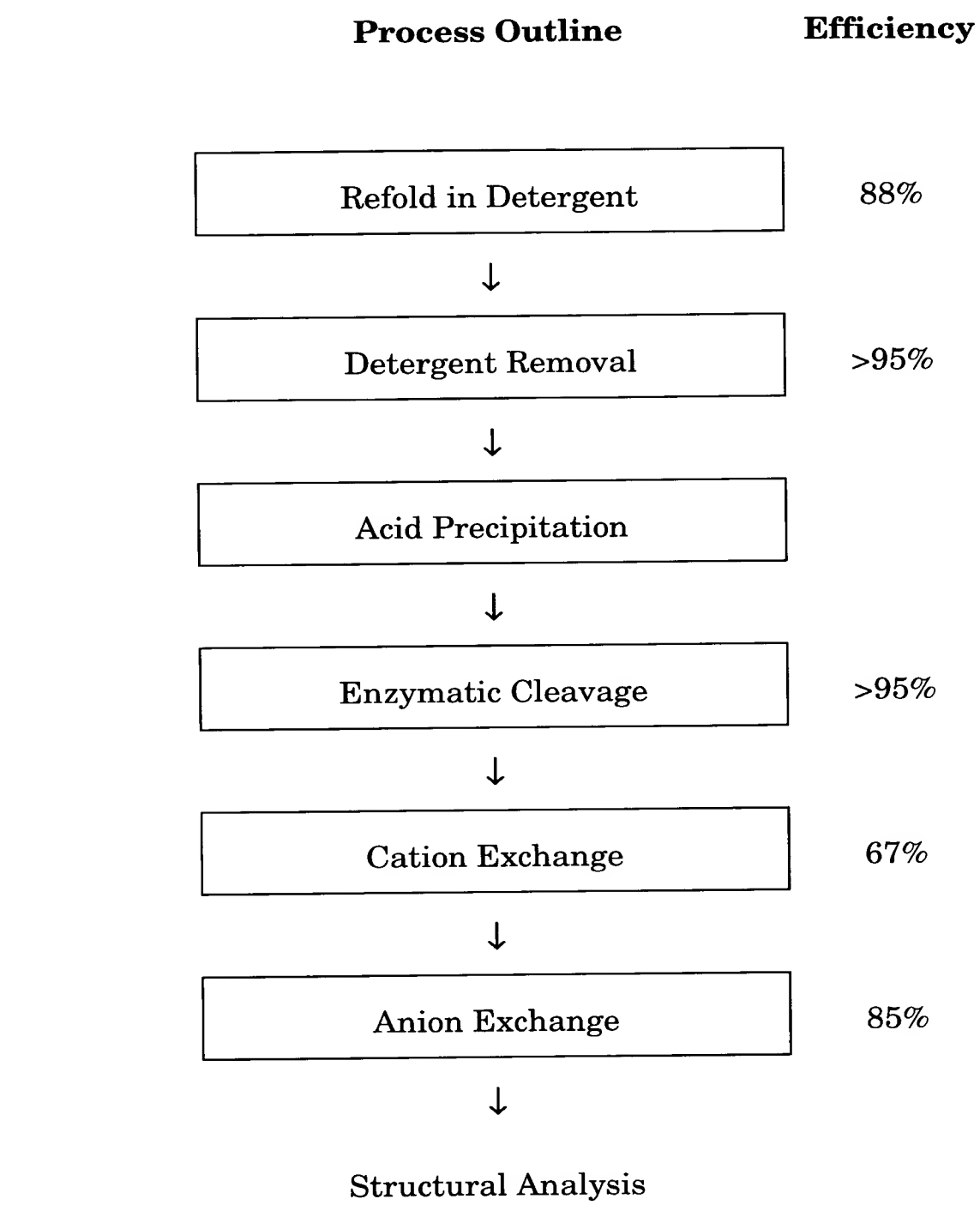
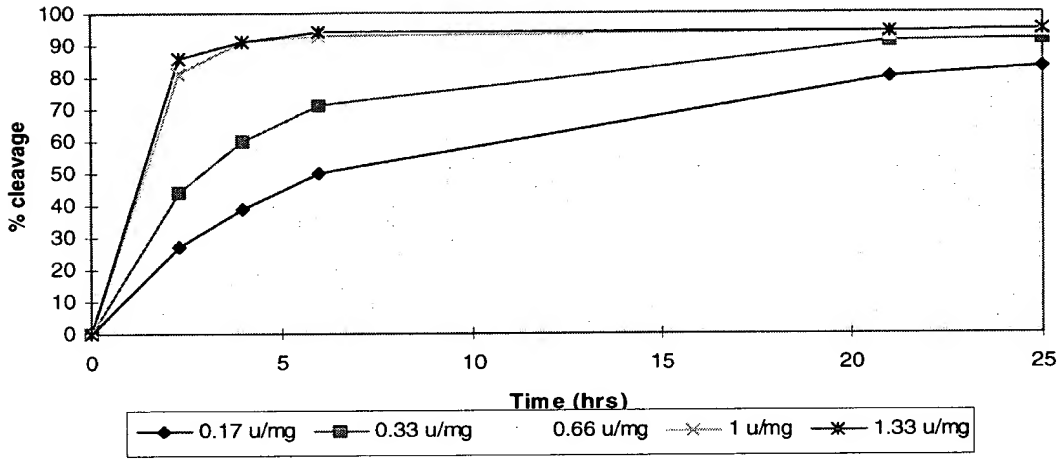
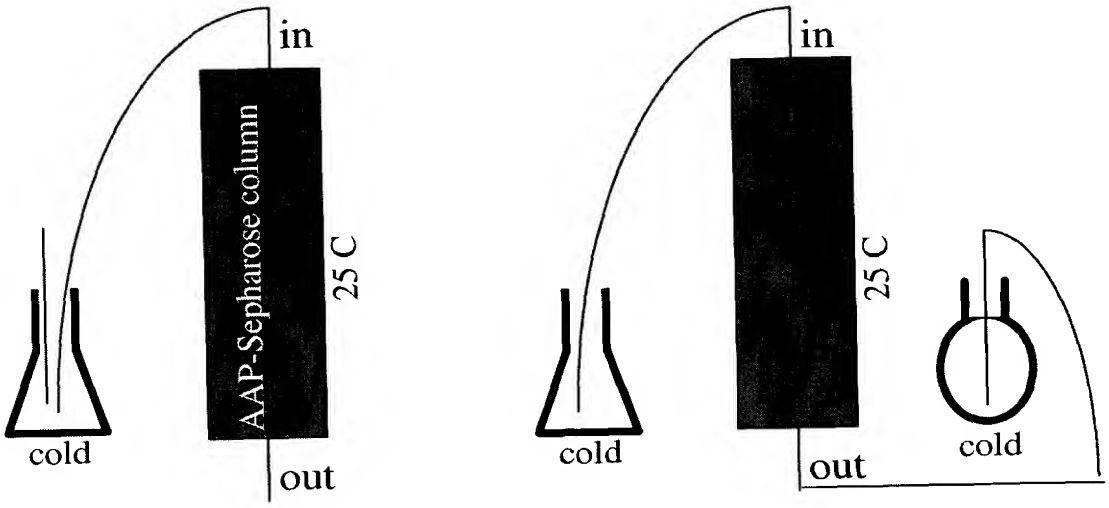


Figure 3 **- Kinetics of removal of Ala from Ala-hGH by AAP**



Studies of enzymatic removal of alanine from Ala-hGH: [Ala-hGH] 3 mg/ml; [AAP] 0.5, 1.0, 2.0, 3.0, and 4.0 units/ml; volume 6 ml. Cleavage efficiencies were determined by ES/MS. All reactions were performed at room temperature.

Figure 4 - Enzymatic cleavage process options - Column mode



Re-circulation mode
192 ml/hr;10 passes/24 hr
Residence time= 180 min

Flow-through mode
19.8 ml/hr; 1 pass
Residence time= 175 min

Figure 5 - Batch mode cleavage of Ala-hGH

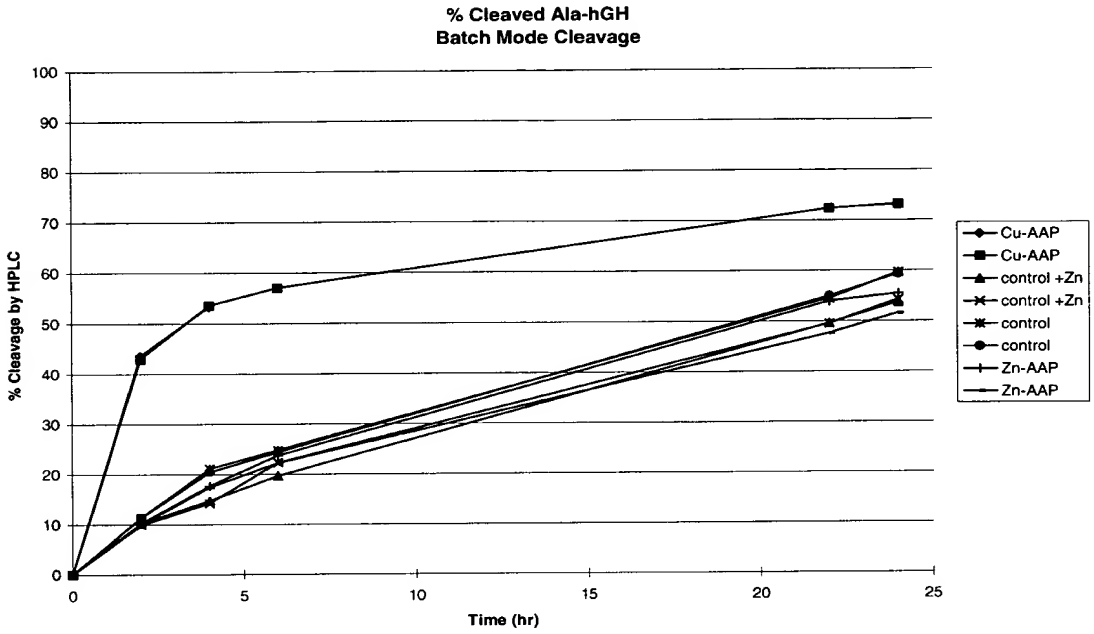


Figure 6 - Recirculation mode cleavage of Ala-hGH

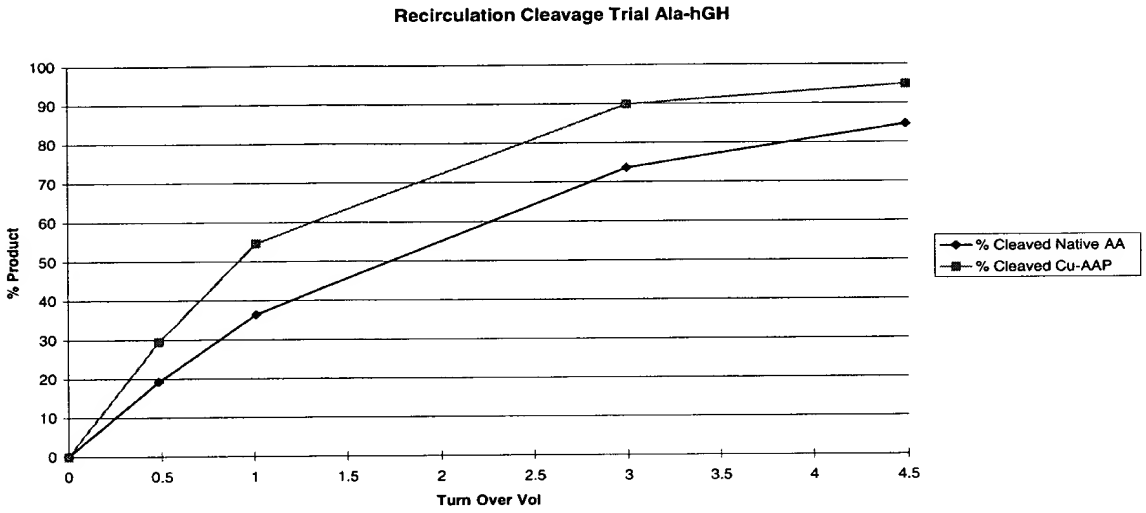


Figure 7 - Cleavage efficiency analyzed by HPLC

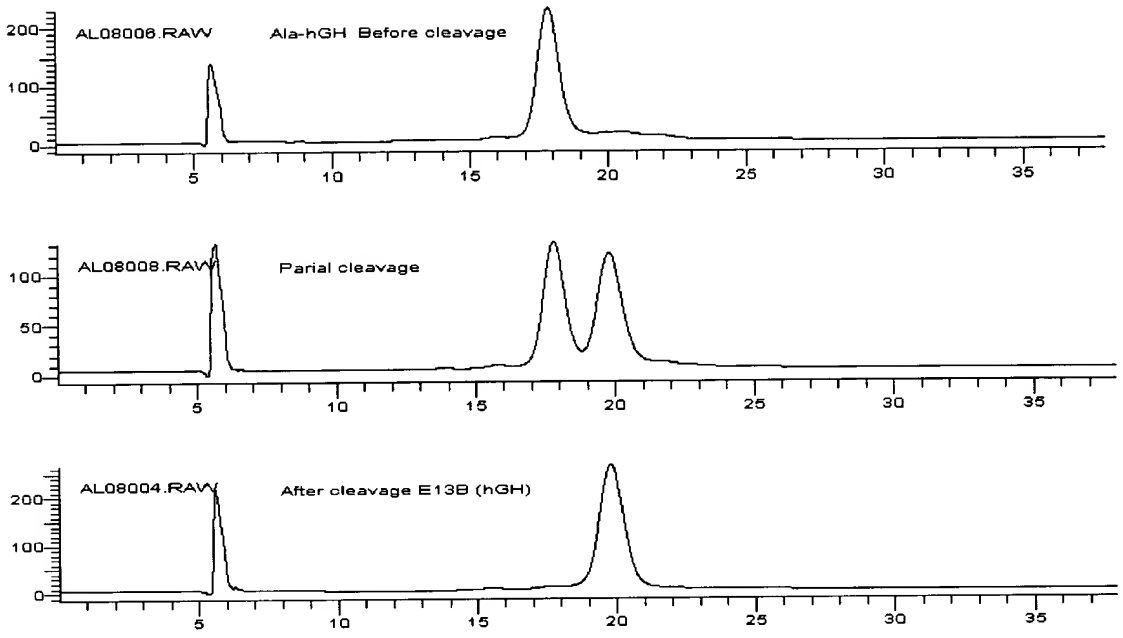


***Figure 8 - Product comparison by* RP-HPLC**

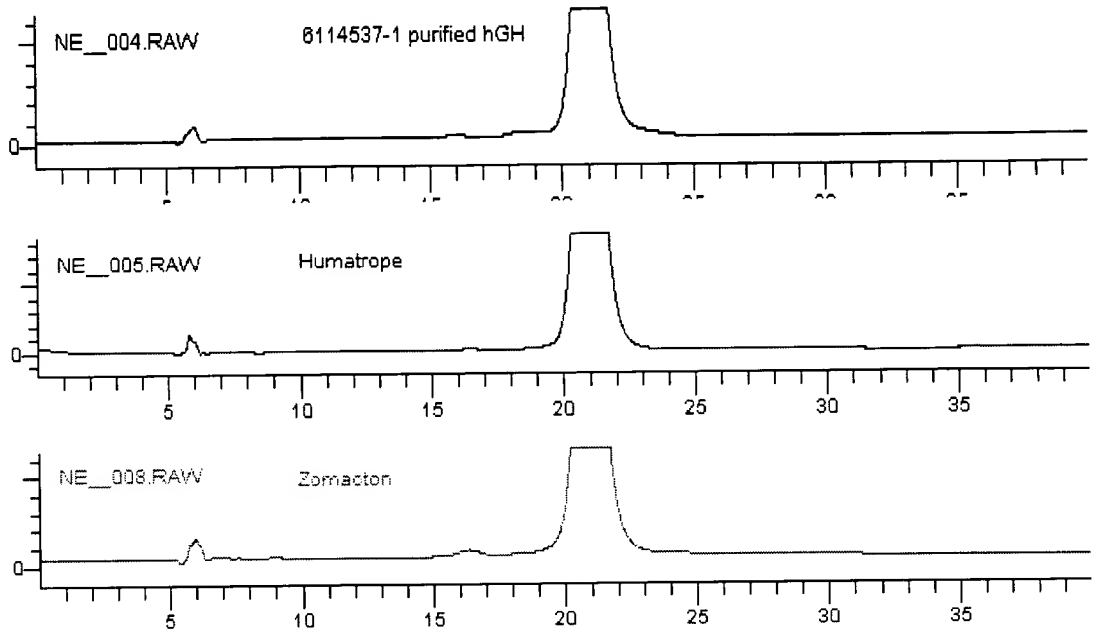
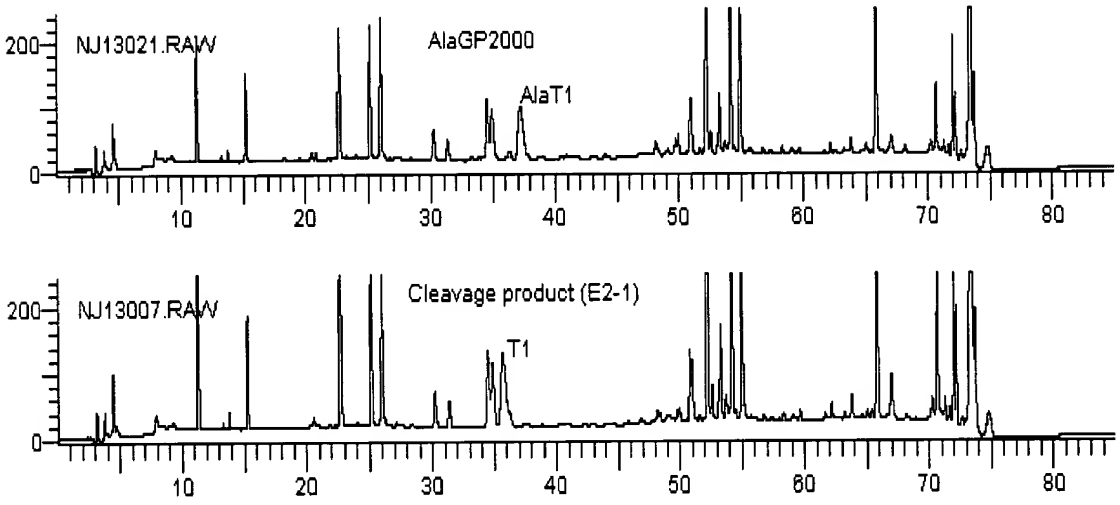


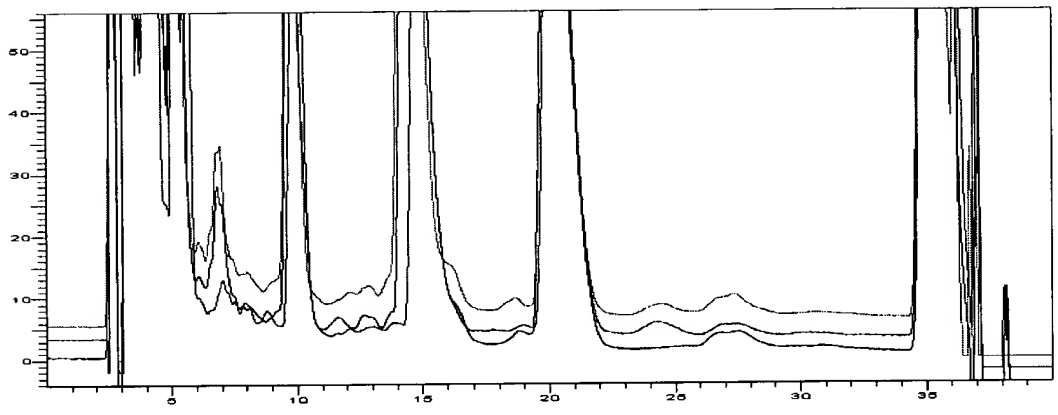
***Figure 9 - Products of Ala-hGH treated with AAP* analyzed by tryptic mapping**



Ala-T1: N-terminal peptide from Ala-hGH (top) (AFPTIPLSR)

T1: N-terminal peptide from hGH (bottom) (FPTIPLSR)

Figure 10 - Quantitative analysis of residual Ala-hGH in hGH by tryptic digest



Baseline resolution of Ala-T1 and T1 peptides allows quantification of residual uncleaved Ala-hGH.

Figure 11 - Product comparison by tryptic mapping

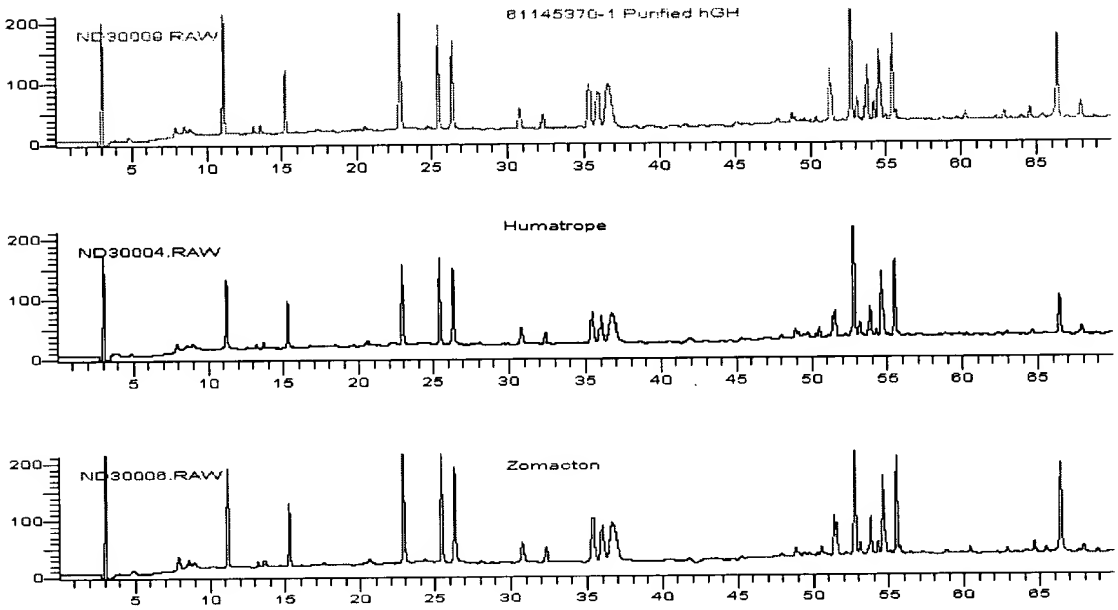


Figure 12 - Residual levels of Ala-hGH in the final product by tryptic mapping

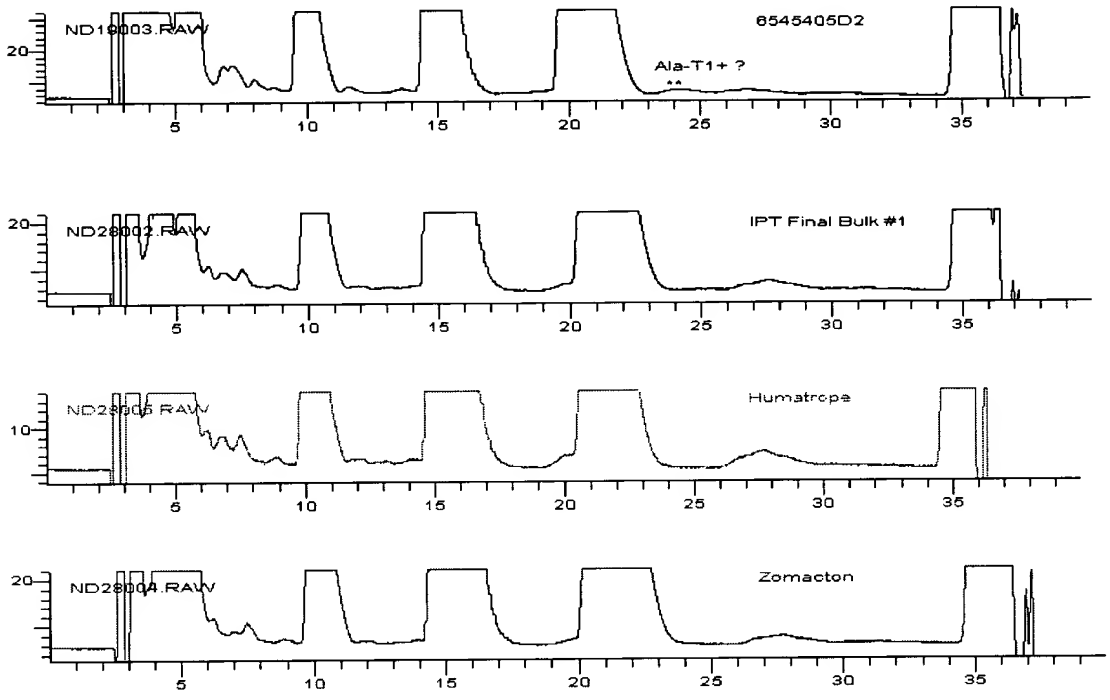


Figure 13 - Cleavage efficiency analyzed by ES/MS

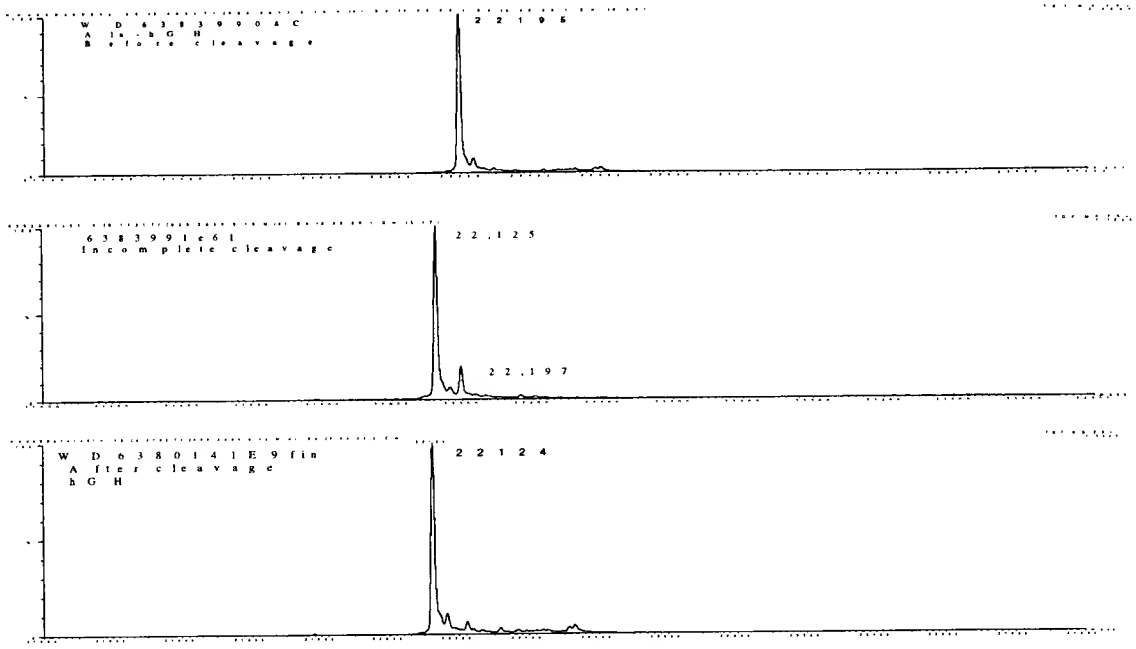


Figure 14 - Product analysis by RP-HPLC, SE-HPLC, and ES/MS

PRODUCT ANALYSIS

Top: RP-HPLC (Vydac C18) using 65-85%/20 min acetonitrile gradient in 1% TF
Center: SE-HPLC (BioRad Bio-Sil SEC 250) using 60% acetonitrile/80 mM TFA isocratic
Bottom: ES/MS: Ala-hGH (22,198); hGH (22,125)

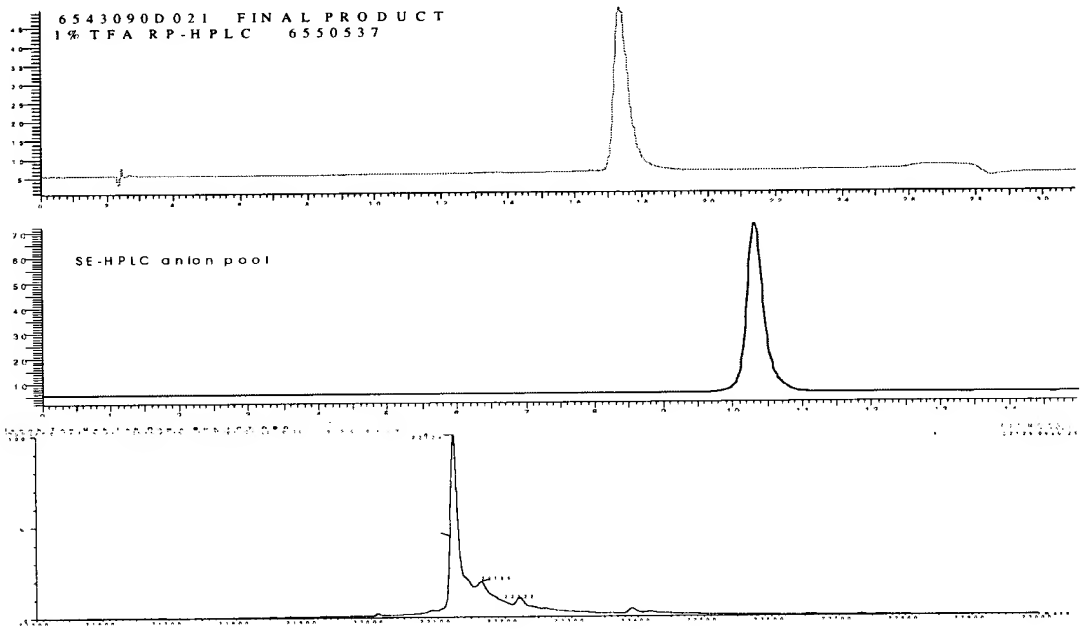


Figure 15 - Product analyzed by N-terminal sequencing

In cycle 1 (left), Phe (18.3 min) is the only significant residue detected; Proline (14.2 min) is the expected residue for cycle 2 (right). The amount of alanine (*, is negligible in both cycles indicating no Ala-hGH in the product.